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Plant Based Pesticides: Green Environment with Special Reference to Silk Worms

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Additional information is available at the end of the chapter

1. Introduction

Pesticides once having entry to an environment either get into the complex web of life through food chain or different components of the environment through physical passages like drifting by air and aquatic runways. Such facts were meticulously described by Rachel Carson [1] in her book 'Silent spring' where she advocated for choosing either the chemical control or biological control to avoid creation of endless problems to mankind owing to pesticide use. Looking back at the history of tremendous potentiality of synthetic chemicals to manage insect pests followed by subsequent cases of failure of chemical control due to development of insect resistance and pest resurgence, here we intend to cite examples of selective toxicity of insecticides and reiterate its importance in management of insect pests. Pesticidal pollution is a global problem. Use of synthetic insecticides to control pest around the world has resulted in disturbances of the environment, secondary pest resurgence, pest resistance to pesticides, lethal effects to non-target organisms as well as direct toxicity to users. It has been reported that about 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches $100 billion annually. The reason behind this amount of cost is the high toxicity and non biodegradable properties of pesticides and the residues in soil, water resources and crops that affect public health [2]. Hence search for the environment friendly, highly selective, newer biodegradable pesticides for pest management programme has been advocated to be essential for last several decades.

South East Asian countries are the hubs for production of raw silks produced by sericigenous insects namely *Bombyx mori* (mulberry silk worm), *Antheraea assama* (Muga silk worm), *Antheraea mylitta* (Tasar silk worm) and *Philosamia ricini* (Eri silk worm). India holds second position in world's raw silk production and contributes to 13.45 % of the total production [3].Yet the production is not sufficient even to meet the domestic demand of the

raw silk. One of the major constraint in silk production is the susceptibility of the silkworms to attack of different pests, parasitoids, predators and pathogens. Even mulberry silkworms which can be cultured in indoor condition are not free from such constraints. In addition, their host plants are also susceptible to the attack by herbivorous pests (insects, mites) and various pathogens (Nematodes, bacteria, fungus and virus) [4,5]. Growth and development of silkworm to a great extent depend on quality and quantity of food consumed and utilized [6]. In such situations, usually farmers take shelter of spurious chemicals for controlling the pest population unless they are well versed with efficacy of pesticides and their hazardous effects. Application of insecticide in sericulture field is not at all advisable as the leaves of host plants are directly consumed by the silk worms and silk worms become affected either through consumption of contaminated food or contact toxicity of the insecticides. *Bombyx mori* are highly susceptible to insecticides and in China its production is reported to be decreasing by almost 30% per annum because of insecticidal poisoning [7]. Fenvalerate-20EC (Sumicidine-20EC), one of the commonly used pyrethroid, reduced the rate of feeding, assimilation and efficiencies of conversion of ingested and digested food into body substance in late instar larvae of *Bombyx mori* [8]. Hexachlorocyclohexane, an organochloride insecticide was reported to cause decrease in fibroin content, pupal and shell weight, adult emergence percentage, fecundity as well as deterioration in quality and quantity of silk thread in *B. mori* [9]. About 50% of normal water intake of eri silkworm (*Philosamia ricini*) was reported to decrease after feeding with leaves of *Ricinus communis* treated with permethrin, a pyrethroid insecticide which might have resulted due to repellency or disruption of feeding physiology [10]. The chemicals used if are phytotoxic reduce the nutritive quality of the leaves. It is globally accepted that complete elimination of pesticide drift is impossible. More often sericulture fields are contaminated by insecticides sprayed into other crop systems in the neighbouring areas. For instance many of the nonmulberry- sericulture fields in Brahmaputra Valley of Assam are situated mostly in close vicinity of stretches of crop fields like paddy and tea. Thus those sericulture fields cannot be expected to be spared of indirect contamination caused by widespread use of pesticides in paddy fields and tea gardens that warns precautionary measures (Plates 1).

Paddy field　　　　Sericulture field　　　　Tea garden

Plate 1. Components of Seri-ecosystem in Assam

2. Pesticides in environment

Introduction of pesticides into a crop system subjects it into a variety of physico-chemical and biological processes which determine the persistence, fate and the ultimate degradation product. Many workers have shown that only a portion of pesticides sprayed onto crops reach their targets, the rest enter the atmosphere by spray drift, volatilization from soil or water, surface runoff, biotransformation by microorganisms, plants, animals, biomagnification through food chain and photodecomposition [11]. One of the major environmental aspect is the effect of sunlight that may lead to various photoprocesses and to photoproducts which are mostly different from parent pesticides in the environmental properties and toxicological significance. The quantum of light energy emitted from the radiation is absorbed by pesticides in environment and this raises the energy state of the molecule, causes excitation of electrons leading to formation or disruption of chemical bonds. Photolysis of pesticides have been studied in water, soil and plant surface. Various sensitizers present in environment such as riboflavin, humic substance etc. absorb light energy and serve as donor of energy to pesticide acceptor and bring about photodecomposition of pesticides. Most organophosphates whose photochemistry have been studied are phosphorothioate and phosphorodithiotate compounds. Although not highly susceptible to photodegradation by UV light, malathion degrades to different photoproducts such as malaoxon, malathion diacid, o,o-dimethyl phosphorodithioic acid, o,o-dimethyl phosphorothioic acid and phosphoric acid [12, 13].Some of the compounds viz. malaoxon are more toxic than the parent compound. Insecticides aimed against pest population may enter non-target arena through spillage at sublethal level but even these sublethal dosages may exert considerable damage on behaviour and activity of non-target population [14]. Pesticidal effect of insecticides at sublethal dosages may have long term effect and they may be expressed at a later part of the insect's life [15,6].Continuous exposure to sublethal dosages on the environment may on the otherhand help a pest to develop resistance mechanism against the toxic compound. Troitskaya and Chichigina [16] showed that combined use of bacterial and chemical insecticides in silk-producing areas possess a real danger to *Bombyx mori*.

2.1. A study was carried out to evaluate the susceptibility of the silkworm, *Antheraea assama* to sublethal dosages of organophosphorous chemicals, malathion and phosphamidon in terms of certain developmental and biochemical parameters. The insecticides were sprayed to leaves at sublethal concentrations based on LD50 values determined earlier and allowed the fifth instar larvae to feed on them in 12 D: 12L photoperiodic condition. The parameters considered were mean larval growth rate, food consumption and utilization computed by the method of Waldbauer (17). In case of malathion treatment although at the lowest dose applied the mean daily consumption, utilization and mean larval weight did not vary significantly they decreased at higher dosages. The correlation coefficient between the mean daily consumption and utilization of food and concentrations of insecticide was -0.826 and -0.812 respectively. This represented a feeding deterrent effect of malathion and its ability to

interfere with digestive physiology. Accordingly the growth rate and in later part of developmental period, the percentage of pupation and adult emergence decreased even at lower concentration. In case of treatment with phosphamidon, the daily food consumption and utilization decreased but they did not vary at the highest dose. The correlation coefficient between the mean daily consumption and concentrations of phosphamidon were +0.539 which might indicate probable absence of feeding deterrence ability in phosphamidon. The growth rate decreased, but the mean larval weight increased with increase of concentration and the correlation coefficient between larval weight and concentrations of phosphamidon was +0.930. Although variations were observed in effect of different concentration during larval period, in later stage of development, similarly with the effects of malathion, the percentage of pupation and adult emergence decreased significantly (Figure.1). The mechanism in which the two insecticides interfered with the insect's body physiology was probably different. In order to study the effect of LD40 dosages of malathion and phosphamidon on tissue weight and different bioconstituents, the early instar larvae were allowed to feed on treated leaves and grow till adult. Analysis in late fifth instar larvae before silk spinning revealed that the tissue weight decreased along with total lipid, protein, Glycogen (female) and cholesterol (male) (Figure 2).

3. Insecticide mechanism of action

The major classes of synthetic pesticides are organochlorines, organophosphates, carbamates and pyrethroides. Preliminary survey revealed that organophosphates and pyrethroides are two of the most common pesticide classes used by common farmers against pests of paddy and other crops and vegetables in Assam. They also belong to the most commonly used pesticide groups in tea gardens. Organophosphates like malathion, phosphamidon and dimethoate even at sublethal dose have been reported to be highly toxic against the larvae of *A. assama* [6]. Organophosphates (OPs) are known to cause inhibition of esterases in silk worms [18]. Carboxylesterases constitute a class of the metabolic enzymes involved in insecticide resistance to OPs, carbamates, and pyrethroids through gene amplification, upregulation and coding sequence mutations [19]. The major function of acetylcholine esterase (AChE) is hydrolysis of the neurotransmitter acetylcholine bounded at cholinergic synapses in the central nervous system of insects [20] and the latter confers target site for susceptibility to orgnophosphorous insecticides which in *Bombyx mori* is reported to act through inhibition of *Bm*AChE1 responsible for expression of acetylcholincholine esterase [19,21,22]. Like DDT, Pyrethroides are axonic poisons. But in contrast to the residual persistence and biomagnification effect of DDT for which its use in agricultural crop has been banned over the globe, pyrethroides are the fastest developing group of modern insecticides primarily because of their effectiveness and safety application [23]. The synthetic pyrethroid deltamethrin (Decis) although is effective against a notorious parasitoid of silk worm, *Exorista sorbillans* with LC50 value at 0.106%, the insecticide has been found to be more highly toxic (LC50= 9×10^{-5})to the larvae of muga silk worm, *Antheraea assama* [24]. Aerial application of organophosphates to agricultural field and their

drifting to nearby mulberry plantation was reported to influence food consumption and utilization of silk worms [25]. Lipophilic insecticides are mainly carried in blood [26] in protein and protein associated forms [27]. From blood, insecticides are redistributed to gastrointestinal system, adipose tissue and brain [28-30]. Toxic effect of insecticides and oil pollutants are reported to cause changes in levels of different bioconstituents and metabolic processes in insects[30-34] finally leading to growth inhibition (Figure.1& 2) [6,15,35].
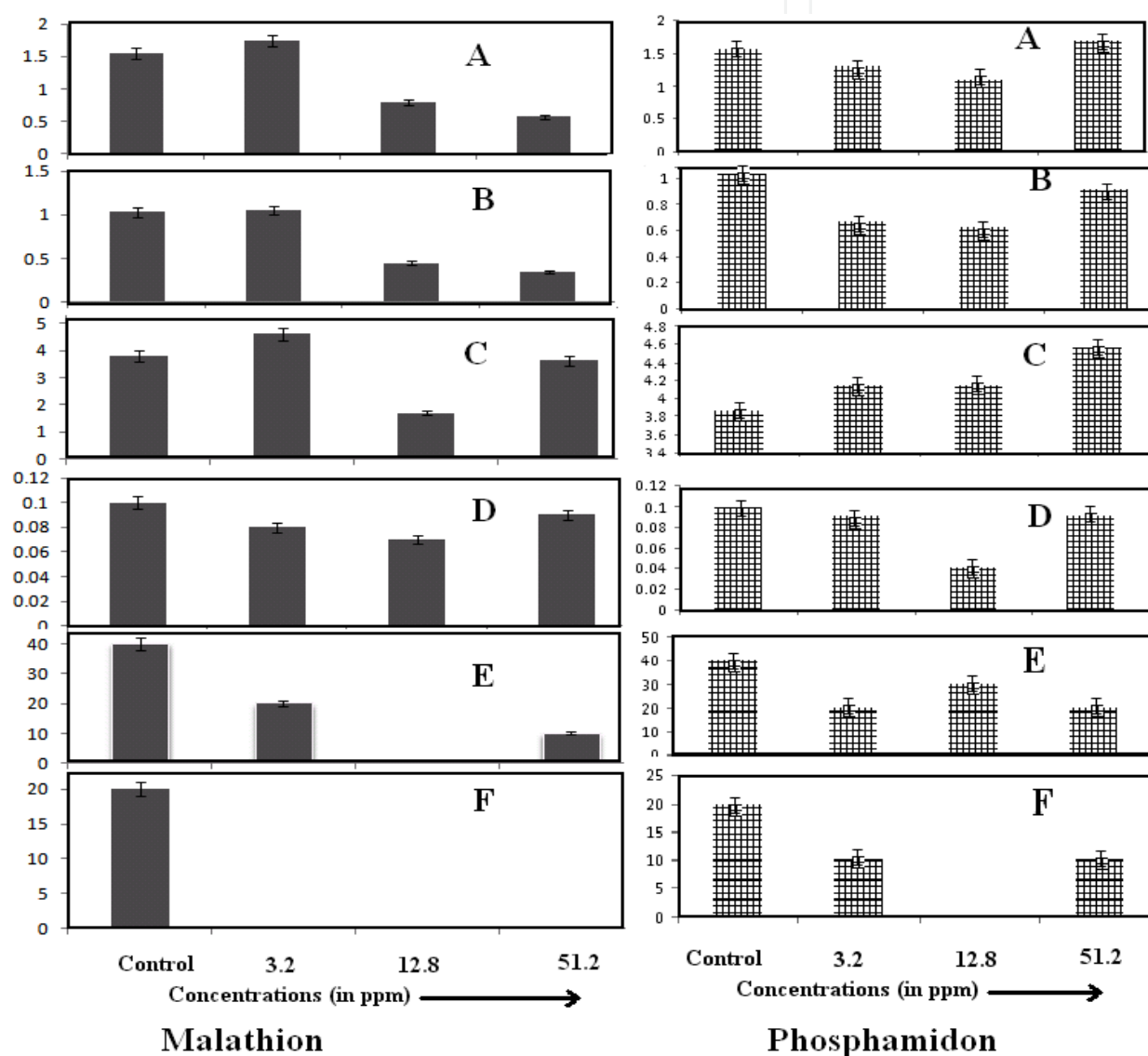
Figure 1. Effect of malathion and phosphamidon on developmental parameters of *A. assama*. A. Daily consumption, B. Daily utilization, C. Mean larval weight, D. Growth rate, E. Percentage of pupation, F. Percentage of emergence
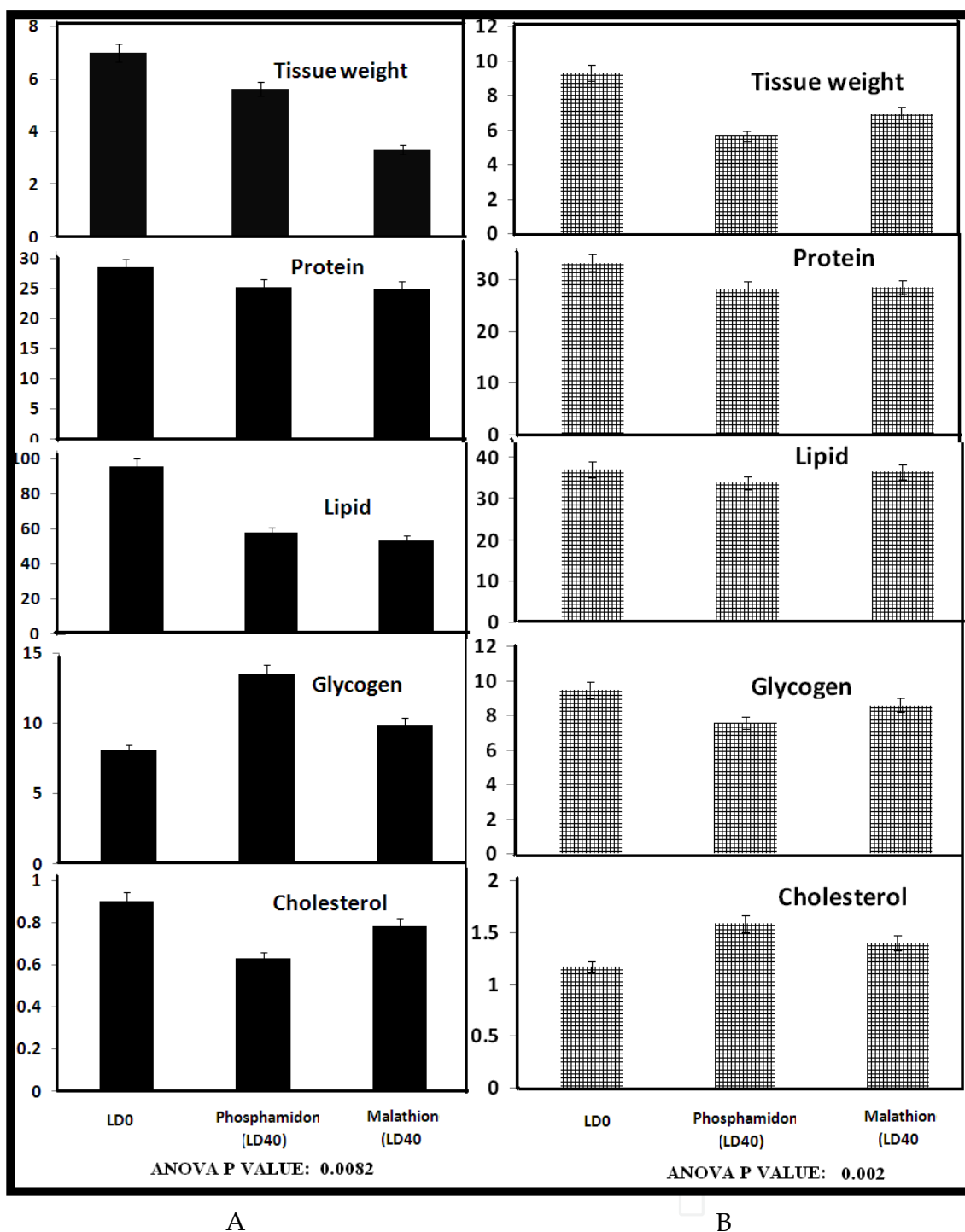
Figure 2. Effect of phosphamidon and malathion on tissue weight and level of biochemical constituents, Tissue weight (mg), Total Protein (mg/weight),Total lipid (mg/ml), Glycogen (mg/ml)and cholesterol (mg/ml).A. Male, B. Female.

4. Natural products

Natural products are excellent alternative to synthetic chemical pesticides. Plants exhibit enormous versatility in synthesizing complex materials which have no immediate obvious

growth or metabolic functions. These complex materials referred to as secondary metabolites are produced as constitutive and induced defense as a result of co evolution arising out of millions of years of plant-herbivore interaction [36-41]. They may be exploited for the management of insect pests owing to their ability to act as toxicant, repellent, antifeedant and insect growth regulators. They are non phytotoxic, biodegradable and have little or no mammalian toxicity [42,43]. Plant extracts and essential oils come under the category of " Green pesticides" as they are safe, eco friendly and more compatible with environmental components compared to synthetic pesticides. 20th century witnessed an increasing trend in use of botanicals with more than 2000 bioactive plant species identified for their insecticidal and anti - pathogenic properties [39,40,44,45]. Natural insecticides such as pyrethrum, rotenone and nicotine have been used extensively for insect control [39]. Limnoids such as azadirachtin and gedunin, present in species from Meliaceae and Rutaceae are recognized for their toxic effects on insects and are used in several insecticide formulations in many parts of the world [46,47]. Neem formulations have been found effective against the mulberry hairy caterpillar, *Eupterotemollifera* Walker [48]. Pink mealy bug, *Maconellicoccus hirsutus* (Green) is one of the major pest which infests the mulberry plants and cause Tukra diseases leading to qualitative loss of leaves. Leaf extracts of *Andrographis* (99.0%), *Leucas* leaf extract,NSKE (99.0%), *Vitex* leaf and *Ocimum* (90.1%) have been reported to act as repellent against the bug[49].

Grasserie (viral), Flacherie (bacterial), Muscardine (fungal) and Pebrine (protozoan) are four common diseases of silkworm and they have been causing heavy loss to silkworm crops in silk producing countries like India and China. Herbal extracts have been tried for control of these diseases. Isaiarasu *et al.* [50] reported efficacy of aqueous and alcoholic crude extracts of *Acalypta indica*, *Ocimum sanctum* and *Tridax rocumbens* against flacherie and muscardine diseases in silkworm. The alcoholic extract of the plant *Tridax procumbens w*ere reported most effective followed by alcoholic extract of *Ocimum sanctum* and *Acalypta indica.* The reported zone of inhibition (area mm2) of *Acalypta indica*, *Ocimum sanctum* and *Tridax procumbens* at a dose of 50 μl against flacherie causing bacteria were 29.9± 9.7, 42.4±6.8, 74.6±6.6 respectively and against muscardine causing fungus were 13.1±6.3, 23.6±3.4, 23.1±9.1 respectively. Aqueous extracts of thirteen plants were tested against cytoplasmic polyhedrosis virus (AmCPV) in tasar silkworm, *Antheraea mylitta* and out of them 2% concentrations of *Aloe barbedensis* (AKP 3), *Psoralea corylifoilia* (AKP13) and *Bougainvillea spectabilis* (AKP 9) were reported effective in suppressing virosis. They reduced mortality of larvae due to virus infection of 66.17%, 64.47% and 57.19% respectively. Total haemocyte count and haemolymph protein were also reported to increase in treated larvae which is considered as expression of immune response against the attack of pathogen [51]. Extracts of *Terminalia chebua h*as been recommended against a potent bacterial strain, *Pseudomonas aerugenasa (*strain AC3) causing flacherie disease in *Antheraea assama* [52].

4.1. Plants of ethnic importance

Indigenous knowledge (IK) is unique to a particular culture and society. IK is embedded in community practices, institutions, relationships and rituals [53]. It forms the basis for local decision-making in agriculture, health, natural resource management and other activities

and constitutes an important component in the global knowledge system. In most cases, IK is an underutilized resource in the development processes [53]. Learning from indigenous knowledge of specific communities used for generations after generations can improve the understanding of their local conditions and saves time, effort and money besides constituting the foundation for activities designed to address regional and global problems. Thus, the natural products based on the indigenous use of botanicals could be one way of mitigating the problems associated with inappropriate use of synthetic chemicals [54].

4.2. Botanicals in sericulture field

In this chapter we restrict our discussions to candidate plants for being used against a parasitoids of silk worm. In sericulture field, farmers of Assam traditionally sprinkle extracts of tulsi (*Ocimum sanctum*) leaf over egg bunches (Kharika) of *A. assama*. No other plants were recorded to be used against pests and pathogens of this silkworm in a survey carried out in Upper Brahmaputra valley of Assam during 2007-2011. Uzi fly, *Exorista sorbillans* (Diptera: Tachinidae) is a parasitoid of silkworm and a serious threat to sericulture industry. The mated adult female fly lays eggs on the integument of third to fifth instar larva of *Antheraea assama*. The maggots after hatching pierce through the integument and grow inside the body of the silk worm by feeding on the fat body. The matured maggot pierce through the shell of the silk cocoon and crawl away from the site of the cocoonage in search of suitable place for pupation (Plate 2). The piercing of the cocoon shell renders the silk cocoon unreelable. In this way, the fly is reported to cause 20-80% loss of seed crop of *A. assama*. The fly infestation is reported in all the commercial silkworm varieties and from almost all silk producing countries of the world [55,56]. A total of ten plants possessing insecticidal and medicinal values including *O. sanctum* were bioassayed against *E. sorbillans* (Table 1). Leaves of the plants were shade dried, ground to fine powder and extracts were prepared by using ethanol, water and hydroalcohol (50:50). Ethanolic extract of three plants namely *Catheranthus roseus*, *Ocimum sanctum and Ageratum conyzoides* proved effective by causing 53.33 %, 22.17%, 57.41% mortality after 24h at 10% concentration. The order of toxicity was *Ageratum conyzoides > Catheranthus roseus> Ocimum sanctum> Melia azedarach> Paederia foetida> Eupatorium odoratum> Polygonum hydropiper> Vitex negundo=Leucas aspera*.

Further fractionation of ethanolic extract of the effective plants using an eleutropic series of solvents viz. petroleum ether, chloroform, butanol and water followed by subsequent bioassay showed that the petroleum ether extract of *Catheranthus roseus*, *Ocimum sanctum* and *Ageratum conyzoides* caused 46 %, 6.67%, 86.21% respectively after 24h and 100%, 33.79%, 100% mortality respectively after 48h at 5% concentration. Results of other solvent fractions were found negligible. The LC50 value of the most effective petroleum ether extract of *Ageratum conyzoides* was recorded as 0.74 percent [57]. Qualitative phytochemical studies of petroleum ether extract of the plants showed positive results for the presence of flavonoids, alkaloids, phenols and terpenoids. *Ocimum sanctum* has been reported to be effective against other Dipterans and many other pests and microorganisms[58-61]. In addition to *Ocimum* the other two plants found effective were *Ageratum conyzoides* (Asteraceae) and *Catharanthus roseus*(Apocynaceae). Extracts of different species belonging to Asteraceae were earlier found effective against mosquitoes. *Ageratum conyzoides* with a

long history in medicinal use is a common weed species and naturally grows in abundance in Assam. It is widely distributed in tropics and subtropics and has been used in various parts of Africa, Asia and in South America for curing various diseases like purulent ophthalmia, ulcers, wound caused by burns, asthma, dyspnea, pneumonia and also as purgative, febrifuge, antiinflammaory, analgesic, anti-diarrheic etc. Both essential oil and the major component precocene have antijuvenile hormone activity on a variety of insects [62]. In addition to their medicinal importance *Catharanthus roseus* has been reported effective against many insect pests including Dipterans. Volatile components obtained by hydrodistillation was found to contain 76 compounds [63].
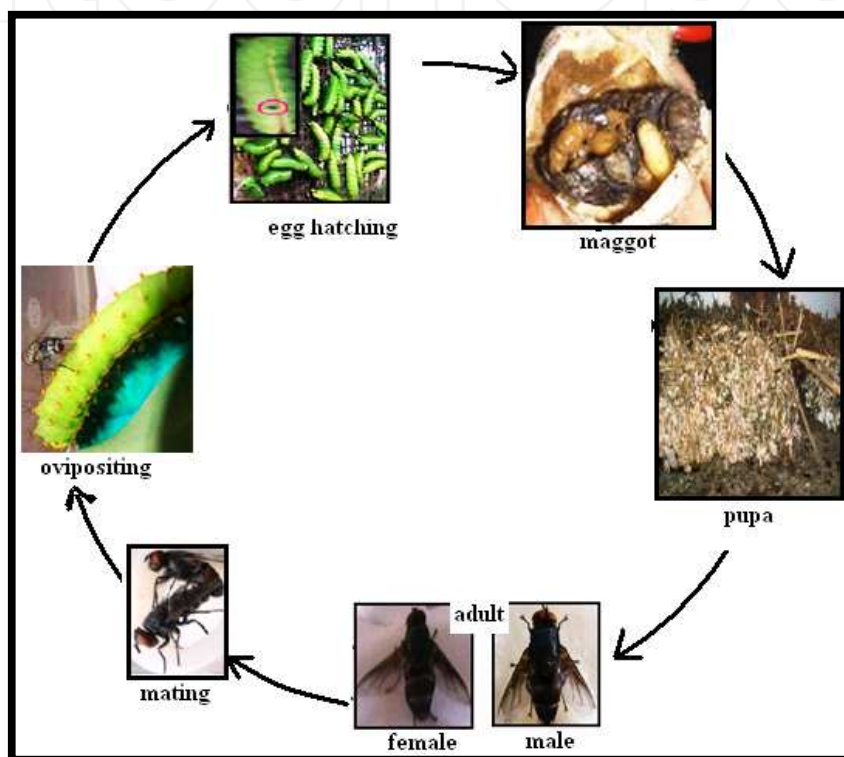

Plate 2. Life Cycle of *Exorista sorbillans* (Uzi fly)

Sl. No.	Plant	Cold water (Mean±SE)	Hot water (Mean±SE)	Hydroalcohol (Mean±SE)	Ethanol (Mean±SE)
1.	*O.sanctum*	0±0	3.33±3.33	3.33±3.33	22.17±4.54
2.	*O.gratissimum*	0±0	13.33±3.33	6.67±3.33	15±5.01
3.	*A.conyzoides*	0±0	10±5.78	13.70±3.17	57.41±4.31
4.	*C.roseus*	0±0	3.33±3.34	3.33±3.34	53.33±21.88
5.	*E.odoratum*	0±0	3.33±3.34	0±0	3.33±3.34
6.	*L. aspera*	0±0	0±0	0±0	0±0
7.	*M.azedarach*	0±0	0±0	0±0	6.67±3.3
8.	*P.foetida*	0±0	10±0	0±0	3.33±3.34
9.	*P.hydropiper*	0±0	3.33±3.34	0±0	0±0
10.	*V.negundo*	0±0	0±0	0±0	0±0

Table 1. Comparative toxicity of crude extracts of plants (10%) to *E.sorbillans* (contact residual film technique)

4.3. Botanicals in paddy cultivation

Farmers in some locations of Brahmaputra valley of Assam use plant materials in rice fields for prevention of pest infestation. For instance, robub tenga (*Citrus maxima*), chuka tenga (*Citrus medica*), jora tenga (*Citrus spp.*), leaf of baghdhaka (*Chromolaena odorata*), patharua bihlongoni (*Polygonum hydropiper*), chirata tita (*Andrographis paniculata*) etc. But, no systematic study has been carried out so far to ascertain if farmers are deriving any benefit or there is any scientific basis behind the beliefs of the farmers. A survey done by interactions with 200 farmers in 44 villages revealed that 23% farmers used synthetic chemical pesticides, 37% used traditionally used plant parts, 27% used both synthetic chemicals and traditionally used plant parts,while 13% adopted neither synthetic chemicals nor traditionally used plant materials (Table 2) [64].

Similarly with the discussions in context with pests and their control in sericulture field, here we limit our discussions regarding traditional use of plants and their scientific validation in context with a representative pest of paddy. *Nymphula depunctalis* (Guenée) [=*Parapoynx stagnalis* (Zeller)] (Lepidoptera; Pyralidae) is a serious pest of paddy that attacks young rice plants in waterlogged paddy fields of the Oriental region [65-70].It also thrives on various other grasses [71]. A number of related species occur in Asia but *N. depunctalis* is most widely distributed occurring in South and South East Asia, China, Japan, Australia, South America and Africa [72-74]. In many parts of Asia, *N. depunctalis* has been reported as a major pest [75-79]. In India, it is one of the serious pests in the states of Andhra Pradesh, Assam, Bihar, Karnataka, Orissa, Tamil Nadu, Manipur and Kerala. Damage to the plant occurs mainly due to defoliation by scraping of the green tissues by the larvae leaving only the white papery epidermis behind. Upon hatching, the first instar larvae climb onto a leaf and begin feeding on the green tissues by scraping the leaf surface. They then move to the leaf tip and cut a slit on the margin at a location 2–3 cm below the leaf tip. Then they make another cut about 1 cm below the first cut. Due to a lack of turgor pressure, the cut leaf segment rolls around a feeding larva to form a tubular case that is secured by silk spun by the larva. The inner surface of the leaf case is lined with silk to hold a thin film of water that is essential for respiration and to prevent desiccation of the larva[80]. Pupal period is spent within a closed case. (Plate 3).

Twenty two plants were found to be used traditionally in paddy fields against *N. depunctalis* and out of them extracts of 13 plants were selected for bioassay against *N. depunctalis*. The methods used for bioassay were residual film technique [81] and case dip technique [82]. The selected plant parts were shade dried, ground to powder and used to prepare extracts using water, hydro-alcohol (50:50) and ethanol. While hot water extract was the least toxic, both hydro-alcohol and ethanolic extracts were highly toxic. Out of thirteen plants tested, ethanol extract of seven plants were found highly effective (90 -100% mortality), five plant extracts were found moderately effective (50 – 89% mortality) and one was found the least effective (mortality less than 50%). The highly effective ones were leaves of *Calotropis procera*, root-bark of *Zanthoxylum nitidum*, leaves of *Zanthoxylum rhesta*, stem bark of *Crataeva nurvala* and leaves of *Croton tiglium*, *Vitex negundo* and *Chromolaena odorata*.

The moderately effective ones were leaves of *Melia azedarach*, *Dryopteris filix-mass*, *Polygonum hydropiper* and *Tephrosia candida*, roots of *P. hydropiper*. Leaf of *Premna latifolia* was found the least effective. The degree of toxicity of the effective plants were *Calotropis procera* > *Zanthoxylum nitidum* > *Zanthoxylum rhesta* using residual film technique. The degree of toxicity in bioassay using case dip technique was *Calotropis procera* > *Zanthoxylum nitidum* > *Zanthoxylum rhesta* > *Crataeva nurvala* > *Croton tiglium* > *Vitex negundo*> *Chromolaena odorata*. Phytochemical analysis for alkaloid, flavonoid, tannin, saponin, sterol and glycosides using crude ethanol extract showed the presence of flavonoid, tannin, saponin and glycoside in leaf extract of *Calotropis procera*; flavonoid, saponin, sterol and glycoside in the leaf extract of *Chromolaena odorata*; alkaloid, flavonoid, tannin and sterol in the stem-bark extract of *Crataeva nurvala*; flavonoid, tannin and sterol in the leaf extract of *Croton tiglium*; tannin and sterol in the leaf extract of *Vitex negundo*; alkaloid and sterol in the root-bark extract of *Zanthoxylum nitidum* and flavonoid, tannin, saponin and glycoside in *Zanthoxylum rhesta*. (Figure 1 and 2)[59].
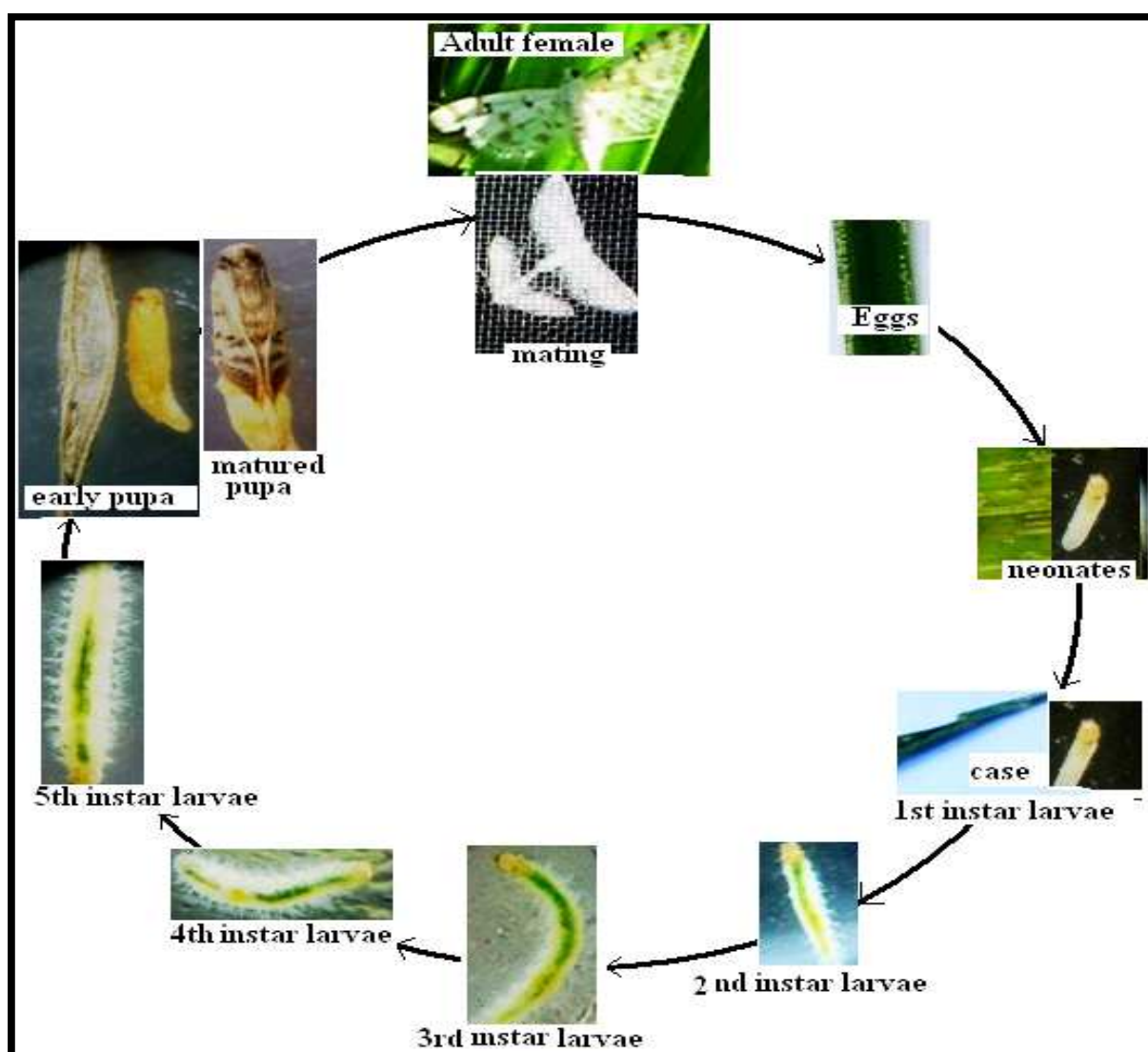

Plate 3. Life cycle of *Nymphula depunctalis*

Scientific Name	Common Name	Family	Part Used	Farmers in %
Ageratum conyzoides	Gundhua ban	Asteraceae	Leaf (LF)*	01.0
Andrographis paniculata	Chirata	Acanthaceae	Leaf	03.0
Azadirachta indica	Mahaneem	Meliaceae	Leaf and Seed	05.5
Calotropis procera	Akan	Asclepiadaceae	Leaf and stem	04.0
Chromolaena odorata	Germany ban	Asteraceae	Leaf	42.0
Citrus grandis, C. medica	Rebab and Bor Tenga	Rutaceae	Fruits and Fruit peel	11.5
Colocasia spp.	Kachu	Araceae	Stem	01.5
Crataeva nurvala	Varun	Capparidaceae	Stem-Bark (SB)*	03.5
Croton tiglium	Koni bih	Euphorbiaceae	Leaf and Seed	03.0
Cympogon nardus	Citronella	Gramineae	Leaf	01.5
Dryopteris filix-mas	Dhekia bilohngoni	Aspidiaceae	Leaf	03.0
Euphorbia neriifolia	Siju	Cactaceae	Stem	00.5
Jatropha gossypifolia	Bhoot ara	Euphorbiaceae	Leaf and Stem-Bark	03.0
Melia azedarach	Ghora neem	Meliaceae	Leaf	05.0
Murraya koeningii	Narasinga	Rutaceae	Leaf	00.5
Polygonum hydropiper	Patharuabihlongoni	Polygonaceae	Leaf, Stem, Root (RT)*	07.5
Premna latifolia	Pitha	Lamiaceae	Leaf	21.00
Tephrosia candida	Kuku mah	Fabaceae	Stem-Bark and Leaf	06.0
Thevetia nerrifolia	Rakta karabi	Apocynaceae	Stem-Bark, Seed	02.00
Vitex negundo	Pasatia	Verbenaceae	Leaf	13.00
Zanthoxylum nitidum	Ricom, Tezmui	Rutaceae	Root-Bark (RB)*	03.00
Zanthoxylum rhesta	Ongare	Rutaceae	Leaf and Stem Bark	02.00

Table 2. List of plants used by the farmers against insect pests of paddy in North East India

4.4. Botanicals in tea gardens

Tea, *Camellia sinensis* (L.) O. Kuntze is grown in a monoculture system and about 1031 arthropod species are reported to be associated with the crop [83]. Cultivation and production of Assam tea was started by Assam company under the British regime around 1840 and the company monopolized the industy till 1860. With the advent of synthetic pesticides and their tremendous potentiality for controlling insect pests, during 50's tea industries started applying insecticides against tea pests which boosted production [83].With the growing concern over pesticidal residue in tea leaves and environmental impact of pesticides, various international regulatory bodies have fixed maximum permissible limit of residues in tea leaves. Synthetic pesticides are still being used in tea plantations although in limited quantity. These pesticides however do not remain confined to the tea plantations only and contaminate the host plants of silkworms grown in sericulture fields lying adjacent to the tea gardens[6]. Literature reveals that several plants have been found to be effective against pests of tea [83-86].
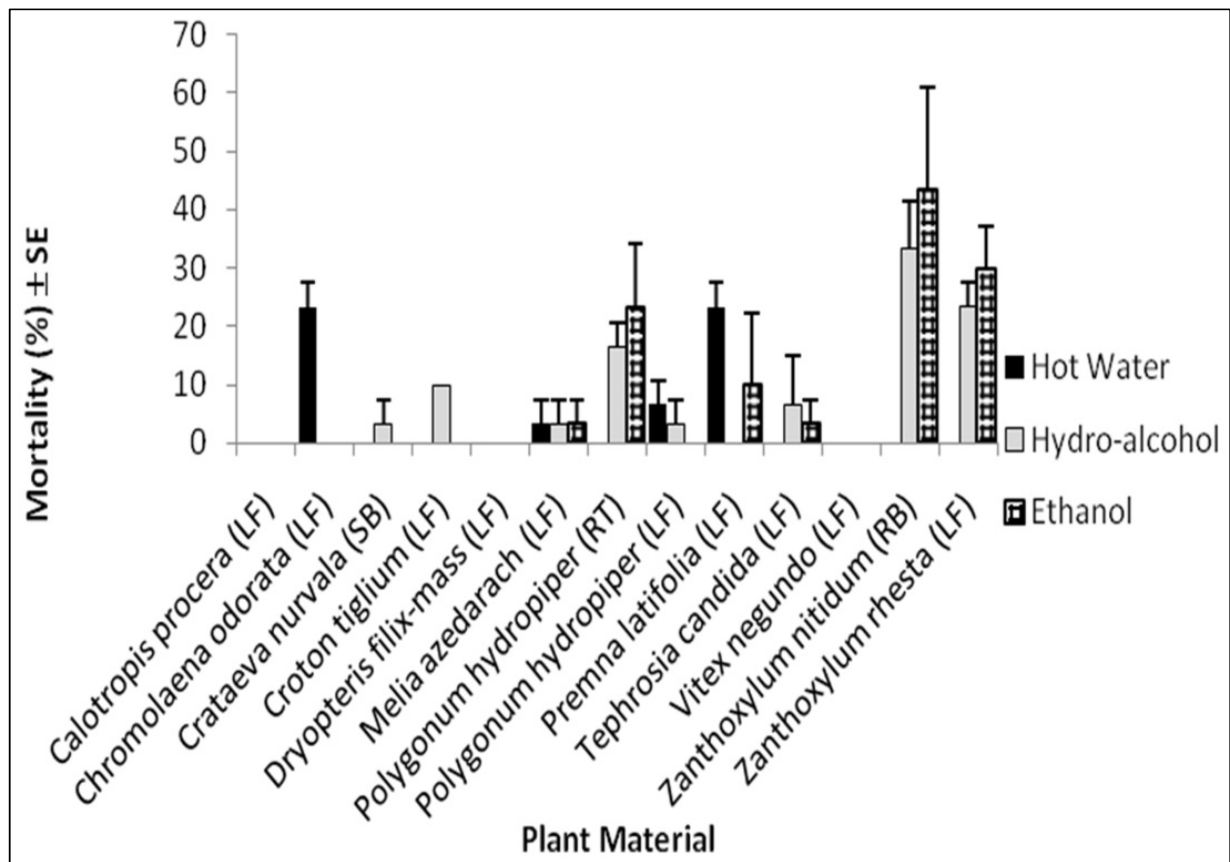
Figure 3. Effects of plant extracts on percent mortality of larvae of *N. depunctalis* in residual film technique
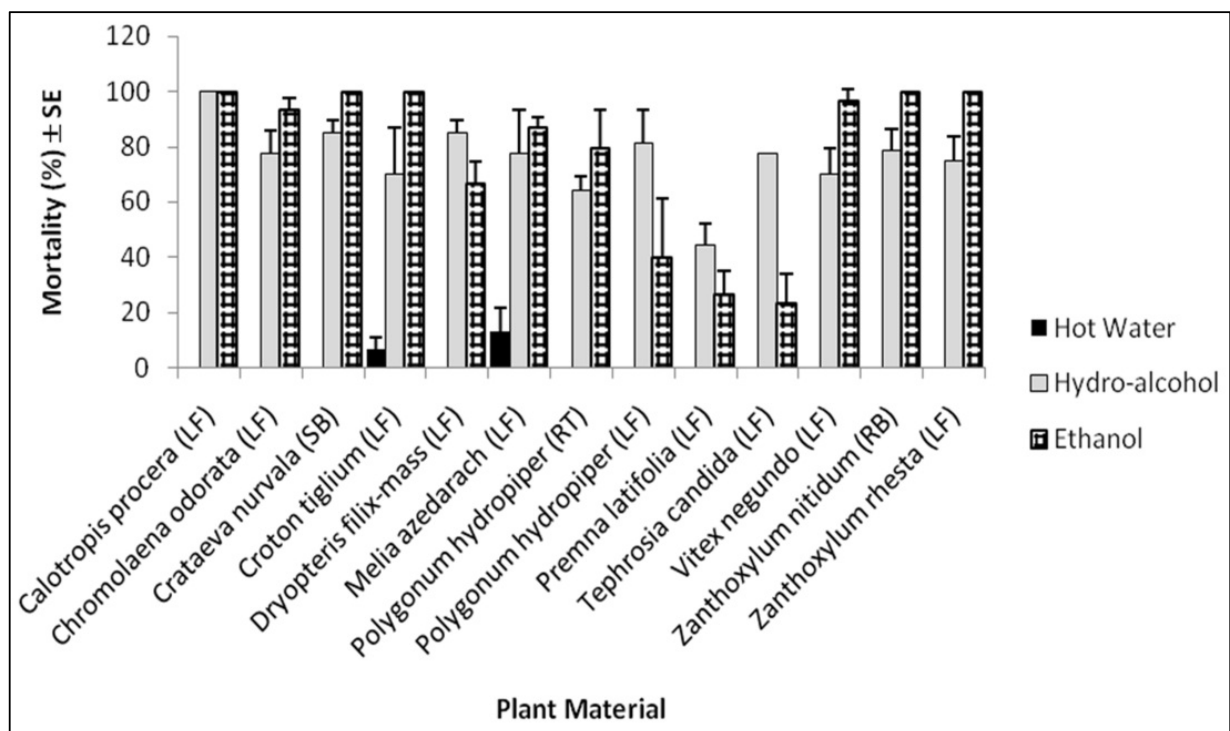

Figure 4. Effects of plant extracts on percent mortality of larvae of *N. depunctalis* in case dip technique.

5. Selective toxicity of plant products

Integrated pest management emphasizes on use of pesticides having selective toxicity as against the use of broad spectrum pesticides. This reminds us of classical examples of using different insecticides for controlling aphids and thrips in potted chrysenthemum in glass houses of Southern Britain in 70's at the advent of development of the concept of integrated pest management [87]. Apart from the aspects why plant products are considered better options for pest control as discussed above, if they can be categorized for having differential efficacy against different insects, the latter can give an added value to plant products for being used in pest control. However the efficacy also depends not only on the compound(s) present in the plant preparation but also on the ability of the insect to defend against the compounds they are subjected to. Less tissue susceptibility, presence of detoxifying enzyme, high immune response, development of alternative physiological pathways, reduced penetration of pesticides through the cuticle and intestine, lower transport of pesticides to the target sites, storage of pesticides in fat body or other inert organs, genetically determined modified behavior in response to pesticide etc. may be factors responsible for conferring resistance to certain insects against high susceptibility of other insect species [23,88.89].

Silk worms have a bitrophic relationship with the Dipteran, *Exorista sorbillans* (Widemann) (Uzi fly) as the later is an endoparasitoid of the silk worm. As silkworms are beneficial insects, before recommending use of any botanical it is desirable to investigate whether the recommended plant or its product found effective against pest and pathogens in seri-ecosystem possess selective toxicity or not. An investigation carried out to evaluate selective efficacy of petroleum ether extract of certain plants against the component insects of the bitrophic system, *Exorista sorbillans and larvae of A. assama* revealed no mortality of late instar larvae of *A. assama* till 48h at 10% concentration while they were highly toxic to the parasitoid *Exorista sorbillans.*

Essential oils are volatile mixtures of hydrocarbons with diverse functional groups. Essential oils are defined as any volatile oil(s) that have strong aromatic components and that give distinctive odour, flavor or scent to a plant. These are the byproducts of plant metabolism and are commonly referred to as volatile plant secondary metabolites. Essential oils are found in glandular hairs or secretory cavities of plant cell wall and are present as droplets of fluid in the leaves, stems, bark, flowers, roots or fruits in different plants. The aromatic characteristic of essential oils attract or repel insect, protect plant from heat or cold and the chemical constituents of the oil act as defense material. Their probable diverse mode of action and use in food and pharmaceutical industry justify their status as possible environmentally benign candidate for pest management. *O.sanctum* and *A.conyzoides* are plants with rich source of essential oils. The toxicity of these oils were evaluated by both contact residual and topical application method against the parasitoid of silkworm, *E.sorbillans*. These oils were found more effective than petroleum ether extract of the respective plants and the calculated LC50 value of essential oil of *O. sanctum* and *A. conyzoides* in contact residual film method were recorded as 0.15% and 0.05% respectively.

But, topical application of the oil showed a different order of toxicity and reflected the fact that degree of toxicity depended on method of application. The Lethal time for *O. sanctum* oil was 4±0.58 minutes and that of *A. conyzoides* oil was 118±12.45 minutes after topical application of 1μl of oil on thorax of the fly and hence degree of toxicity of oil on topical application was *O. sanctum* > *A. conyzoides*.When toxicity of these oils were compared against the larvae of *A. assama*, it was found that late instar larvae could survive till 48h after application of 0.5% concentrations of oil of the plants. The same concentration of oil of *A. conyzoides* while were applied against early instar larvae 100% mortality was caused with LC50 value at 0.19% concentration(Table 3)[81]. But toxicity of oil of *O. sanctum* against *A.assama* larvae was lower and at 0.5 percent concentration, it could only cause a maximum of 5.78% mortality of early instar larvae at 48h of treatment and could cause no mortality in late instar larvae till 48h of observation period (Figure 5). This may suggest less tissue sensitivity of *Antheraea assama* against the action of extract and oil of *O. sanctum* applied [90]. The plant *Ocimum spp.* is known to comprise more than thirty species, but only a few have been subjected to phytochemical studies (Grayer et al. 1996). The whole plant of *Ocimum* is rich in essential oil and based on composition of volatile principles of essential oil, intraspecific chemotypes of several species of *Ocimum* have been described[91-93]. The plant is a part of Indian tradition as a holy substance and its essential oil is larvicidal against both *Aedes* and *Culex* mosquitoes [94].

Plant material	*Exorista sorbillans*			*Antheraea assama*		
	Regression equation	LC$_{50}$ (%)	95% Fiducial limit	Regression equation	LC$_{50}$ (%)	95% Fiducial Limit
A. conyzoides oil	Y=8.72+2.87X	0.05	2.603-3.075	Y=7.10663+2.92014X	0.19	2.394-2.837
O.sanctum oil	Y=9.15452+4.97895X	0.146	8.929-11.172	_	_	_
A.conyzoides petroleum ether	Y=5.17654+1.27665X	0.72	1.220-1.352	Y=3.62422+0.625415X	154.88	0.311-0.937
Deltamethrin	Y=5.90148+.904456x	0.103	1.688-1.969	Y=7.46583+.612381x	.00009	0.572-0.656

Table 3. Efficacy of extract & oil of *A. conyzoides* and *O. sanctum* as compared with Deltamethrin [(_) in cells indicate zero mortality].

5.1. Identification of haemocytes of *A.assama* by light microscopic and transmission electron microscopic studies (TEM) studies

In order to understand the possible effects of the extracts of *O. sanctum* and *A. conyzoides* at cellular level electron microscopic studies of haemocytes were carried out after topical application of the extracts on thoracic surface of fifth instar larvae of *A. assama*.Five types of haemocytes of *A.assama* were identified under light microscopic and transmission electron

microscopic studies. The identified cells were-1. Plasmatocyte 2.Granulocyte 3.Spherulocyte 4. Prohaemocyte 5. Oenocyte (Plate-4-9)
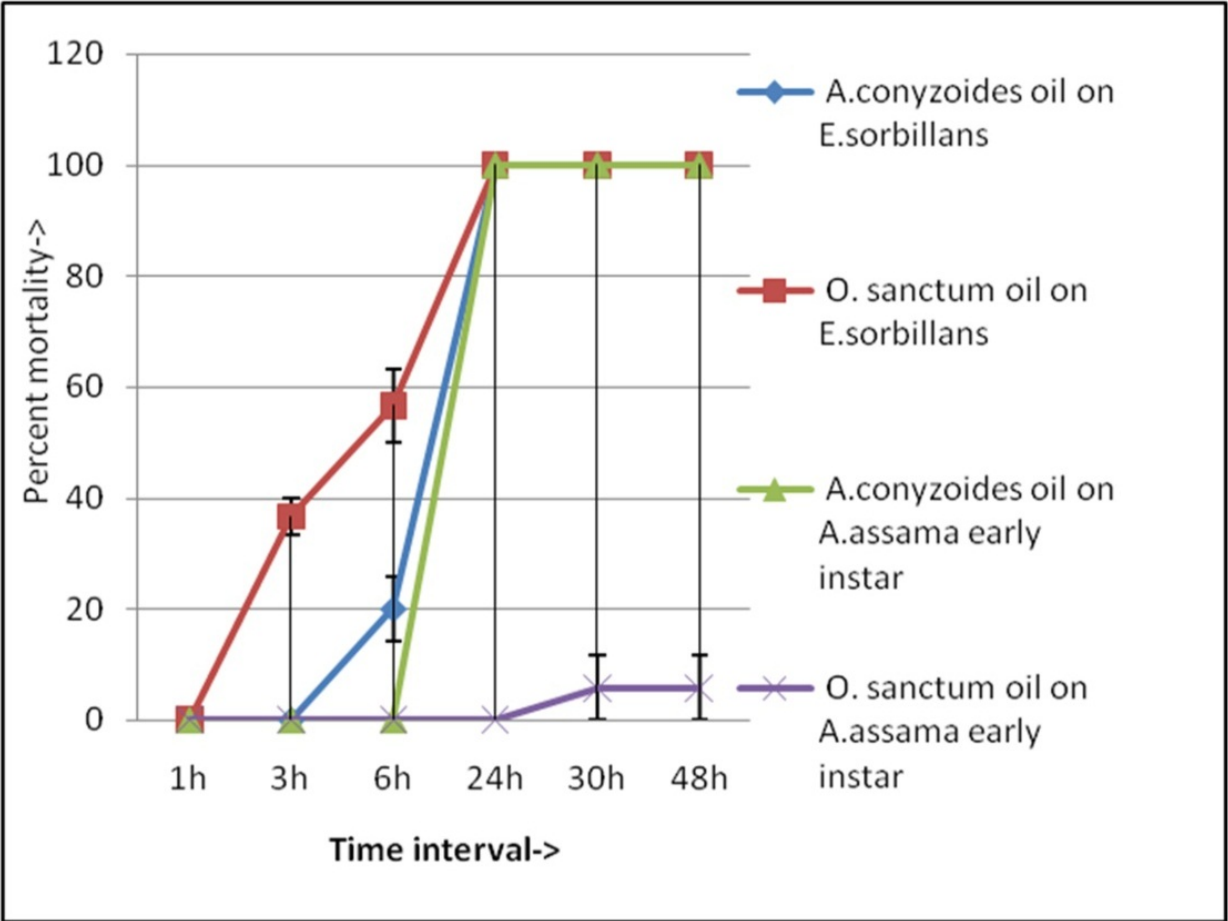

Figure 5. Comparative percent mortality of *A. assama* and *E. sorbillans* at 0.5% concentration of oil of *O. sanctum* and *A. conyzoides*
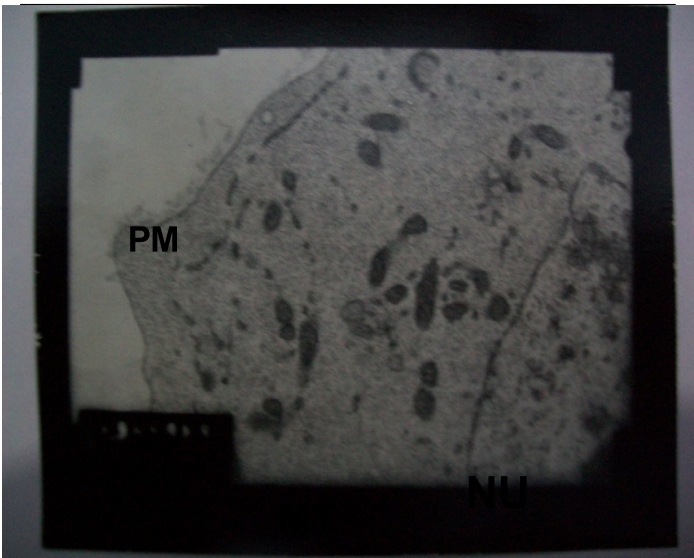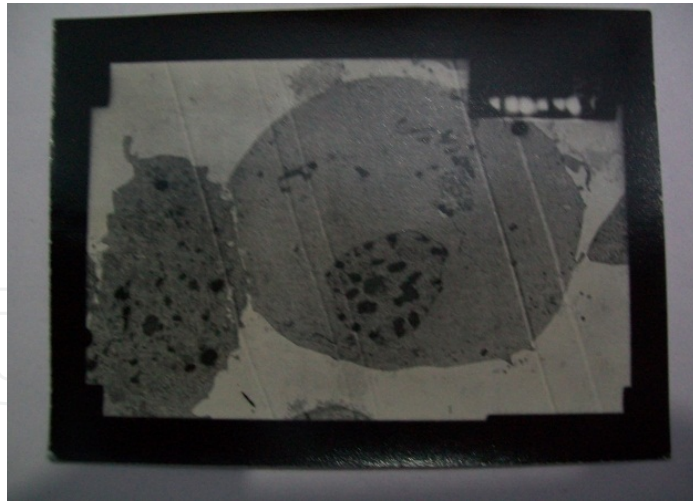

Plate 4. Plasmatocyte in *A. assama*
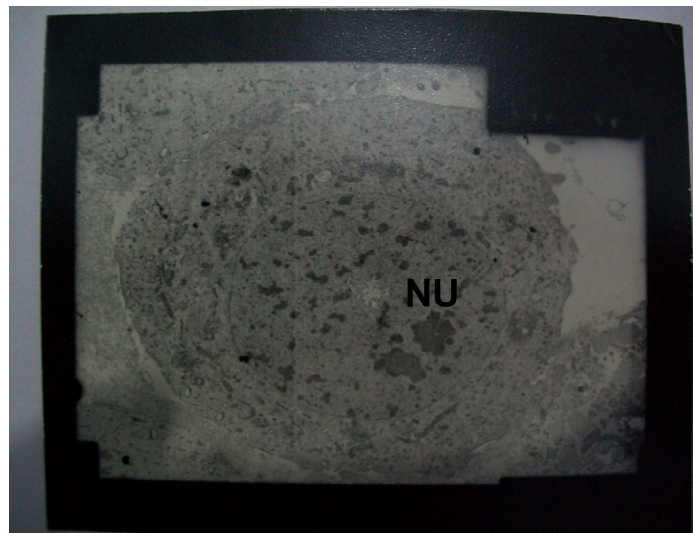
Plate 5. Plasmatocyte and Oeonocyte
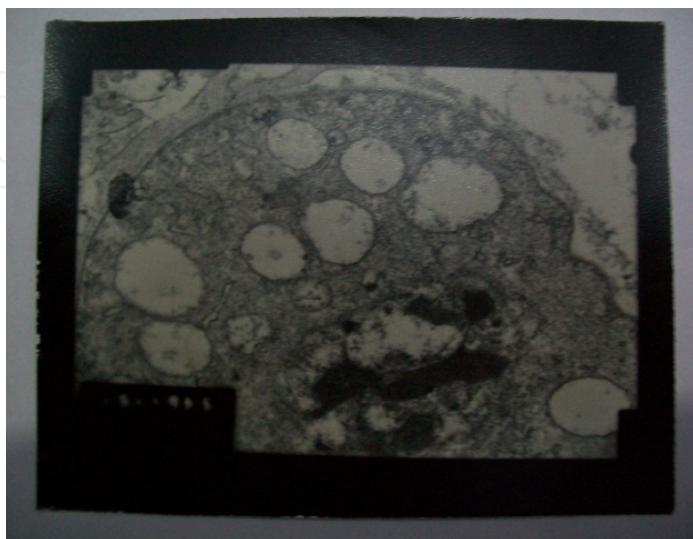

Plate 6. Prohaemocyte of *A. assama*
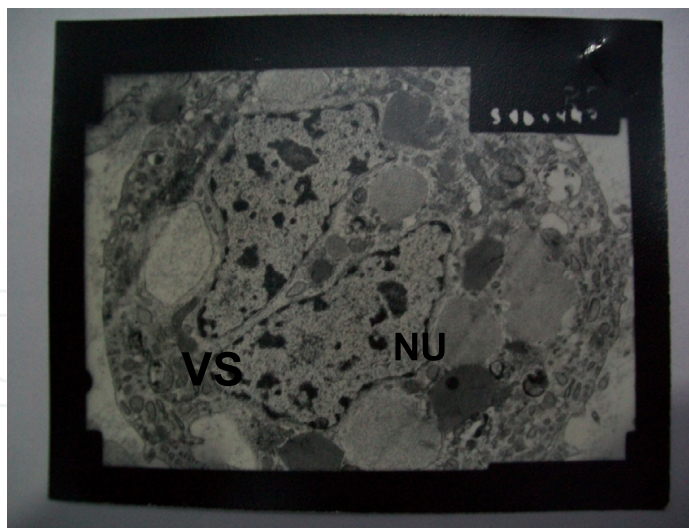

Plate 7. Spherulocyte of *A.assama*

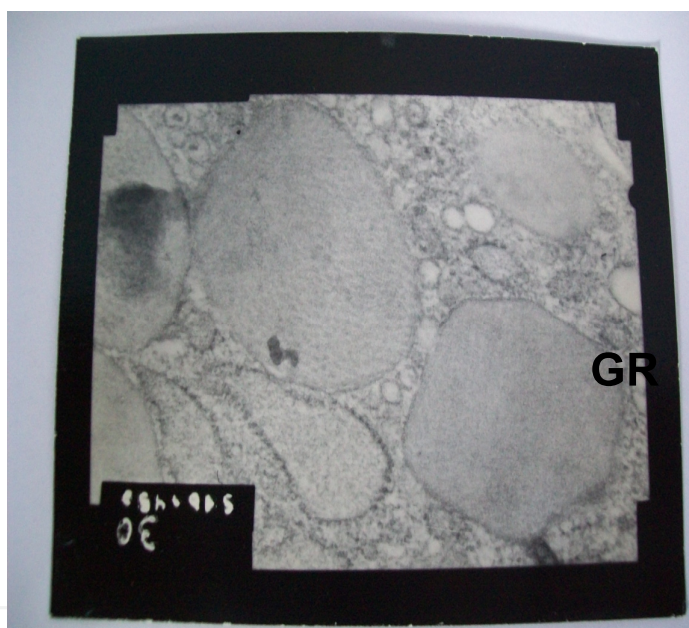**Plate 8.** Granulocyte of *A.assama* under TEM

Plate 9. Structured granule

5.1.1. Plasmatocyte

Plasmatoytes are polymorphic cells with round, ovoid, elongate and spindle shaped structures with filopodia. The cells are small to moderate size. The long axis is 25 μm and the short axis is 14.74 μm. Surface of PLs is not smooth and have ridges much flatter than those of granulocytes. Nucleus is dense, round, ovoid or elongated with distinct membrane. The cytoplasm is generally abundant, granular and agranular. Cytoplasm contains a good number of mitochondria and extensive rough endoplasmic reticulum. Golgi body recorded was in less number. Vacuole is rare in normal cell. The nucleus is mostly centrally placed, prominent, usually round but changes shape with the change in shape of the cell.

5.1.2. Granulocyte

They are rounded or oval in shape with long and short filopodia. The long axis is 8.382 μm. Two types of membrane bound granules were observed, electron dense and structured. Cytoplasm contains large number of mitochondria, endoplasmic reticulum, free ribosomes, lysosomes and small number of vacuoles. Binucleate granulocyte was also recorded. Nucleus generally small takes various shapes, round, ovoid or irregular. The cell surface contains many elevations and depressions giving a conspicuous pattern.

5.1.3. Spherulocyte

These are spherical or ovoid or elongated cells with long axis 7.21 μm. The cell surface contains pits. Wavy edges show presence of spherules. Nucleus is mostly centrally placed, sometimes obscured by spherules. Mitochondria, rough endoplasmic reticulum and golgi complex were observed in cytoplasm when spherule number was less.

5.1.4. Prohaemocyte

They are round, oval in shape and long axis is 7.94 μm. The nucleus occupies major area of the cell. Plasma membrane is almost smooth. Cytoplasm is thin, almost homogeneous, contains few number of mitochondria, Golgi bodies and endoplasmc reticulum. SEM shows a spherical surface.

5.1.5. Oenocytoid

These are large rounded or ovoid cells with eccentric nucleus. Nucleus is small, nucleoli were prominent. The plasma membrane is smooth and regular. Cytoplasm uniformly distributed and contains extensive rough endoplasmic reticulum. Filopods are generally absent. Other cellular organelles like Golgi bodies and mitochondria are less in number.

5.2. TEM studies on effect of petroleum ether extract on haemocytes of late instar larvae

Transmission electron microscopic studies of haemocytes of fifth instar silk worm larvae was done after application of petroleum ether extracts of *O. sanctum* and *A. conyzoides* at different hours of treatment (Plates 10-21).

Application of petroleum ether extract of *O. sanctum* at 0.25 h caused less damage of PL and GR. Mitochondria and nucleus was almost intact in both types of cells. Nuclear membrane and cell membrane damage was negligible. Cell attachment was less in number. Release of material from GR was observed in some points. Golgi body was in formative phase in GR. PL cells in dividing stage and dividing nucleus in GR was recorded.

Cell membrane breakdown of PL was observed at 1h of treatment while mitochondria was intact. Filopods are less. But cell attachment was marked. GR cell was less affected and

filopod like extension was many toward cell attachment site. Granules were released from the area of damage of the cell membrane. Dissolution of nuclear membrane was observed at certain points. OE, SP, PRO was less affected. At 6h of treatment PLwas less affected. Golgi body was observed at formative phase. Filopods are less in number. Export of material from the cell was observed. GR cell was also less affected. Cell membrane, mitochondria were well preserved. Nucleus in dividing stage was recorded. In some GR, granules release from the cell. Some GR attach to one another. Other cells are less affected. But at 12 h of treatment period, cell membrane breakage, mitochondrial damage vacuolization of PL cell was observed while GR cell was less affected. But again at 48h no effect on cell membrane, nuclear membrane, mitochondria was observed. Golgi was found to be in formative stage. Release of material was not recorded. GR cell was looking intact where cell membrane, mitochondria and nuclear membrane were not affected. Aggregation of cell was less. OE cell membrane was ruptured and released some cytoplasmic materials but mitochondria, nuclear membrane were well preserved. Attachment of OE with other cells was observed. SP and PRO were less affected.

At 0.25h of *A. conyzoides* petroleum ether extract treatment, heavy loss of the cell membrane of PL cell was noticed but mitochondrial structure was found almost in good condition. ER long while many Golgi bodies were found in formative stage casting off numerous vesicles. Cell attachment was very pronounced with release of flocculent material. Many vesicles were observed in cytoplasm. Extensive damage of the cell membrane of GR cell was observed. Some of the mitochondria was found in a state of completely dissolved mitochondrial membrane. But nuclear membrane was in good condition. Cell attachment was observed while ER and GB was looking normal. OE was less affected.

At 1h of treatment of *A. conyzoides* petroleum ether extract, damage of cell membrane of PL in some parts were noticed but mitochondria was unaffected. Nuclear membrane was damaged and Golgi body was in formative stage. Filopods present and large vesicles in the cytoplasm was observed. GR cell membrane was found totally damaged state and some of the mitochondria was also affected. But nuclear membrane was less affected. ER short and GB was not observed. Attachment of the cells were observed and numerous vesicles were seen. Other cells were less affected. At 12h of treatment also, damaged PL cell with ruptured cell membrane, affected mitochondria, large vesicles and filopod were recorded. GR cell was also affected with mitochondrial damage, cell membrane rupture, heavy vacuolization. Release of cytoplasmic material to outside of the cell and import of material was noticed.

At 1h of acetone treatment as control almost intact cell membrane, mitochondria, ER, nuclear membrane was observed. Cell attachment was less with few filopods in PL cell. Granules and vesicles number were less and release of cytoplasmic material were not observed in PL cell. But GR cell was affected and plasma membrane rupture and damage in mitochondria was observed. Plenty number of ER but few vesicles were observed. Both types of granules structured and electron dense were observed. Cell aggregation with release of flocculent material was noticed. OE was less affected than PL and GR. But at 6h of acetone treatment PL and GR were found intact with intact plasma membrane, nuclear membrane, normal mitochondria, few vesicles and very less cell attachment. A medium

number of filopods were observed but release of cytoplasmic material was not observed. OE cell was also found intact with unaffected mitochondria. After 48h treatment of acetone also, the nuclear membrane and mitochondria of both PL and GR were found intact with less cell attachment. Cell membrane damage of PL was less. Golgi body was found in formative phase and few number of filopods and vesicles were observed but release of flocculent material was not recorded. Nuclear division of PL cell was observed. Cell membrane rupture of some of the GR cell was recorded with discharge of granule while other cells were intact with almost absence of filopods. Plenty number of ER and large sized vesicles were seen. Both types of granules structured and electron dense were noticed.

A comparative assessment of the effects made showed that the rupture of plasma membrane (PM) in plasmatocyte (PLs) was maximum in treatment with *A. conyzoides* while it was comparatively less in treatment with *Ocimum sanctum.* Symptoms of immune response i.e. cell attachment between PLs and GRs and among the plasmatocytes along with release of cytoplasmic and granular material from granulocytes were observed in case of treatment with all the plant extracts. Toxic symptoms like highly affected mitochondria with break down of nuclear membrane, dissolution of cristae became evident in case of treatment with *A. conyzoides*. In case of treatment with *Ocimum sanctum* the affects were comparatively less and at 48h the PLs were exactly similar with those of control insects. Similar effects on ultrastructure PLs and GRs were reported to be caused in lepidopteran insect by neem gold [95,96]. Plant based pesticides were earlier reported to decrease prohaemocyte, plasmatocyte and spherulocyte and increase granunlocyte and oenocyte population [95,97]. Gupta(1998) reported on involvement of plasmatocytes and granulocyte in cell mediated immune response. In our investigation also cell lysis, release of granular material, vacuolization and clumping of PLs and GRs were observed after treatment with the plant extracts.
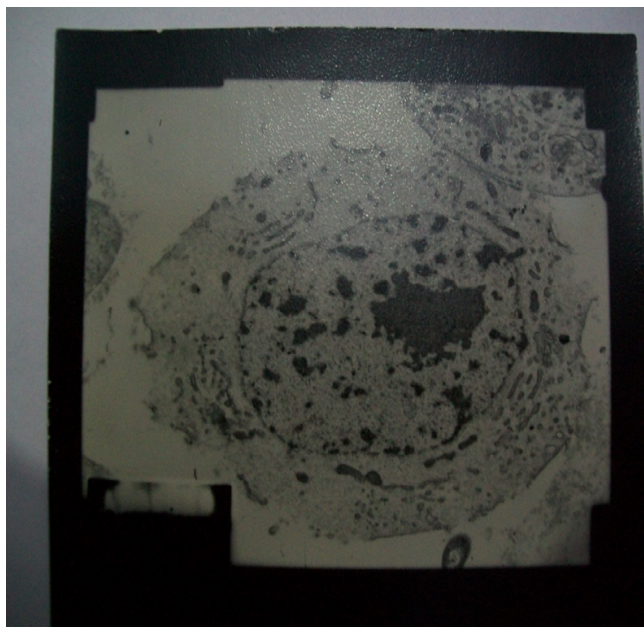

Plate 10. Plasmamembrane rupture in *A.conyzoides* PL under TEM

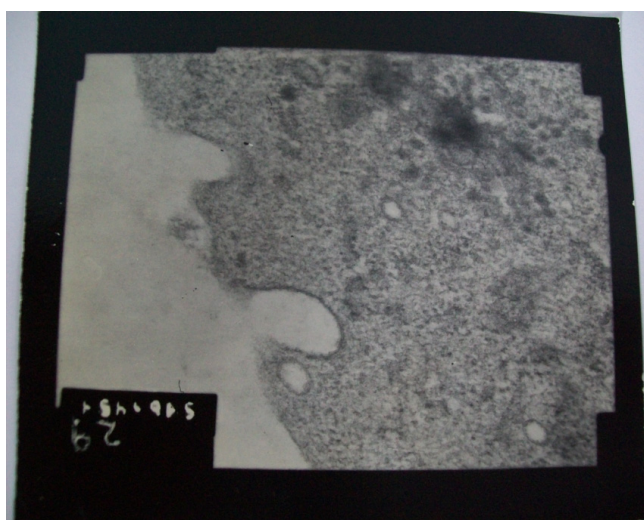**Plate 11.** Nuclear membrane rupture A. *conyzoides* treated PL under TEM

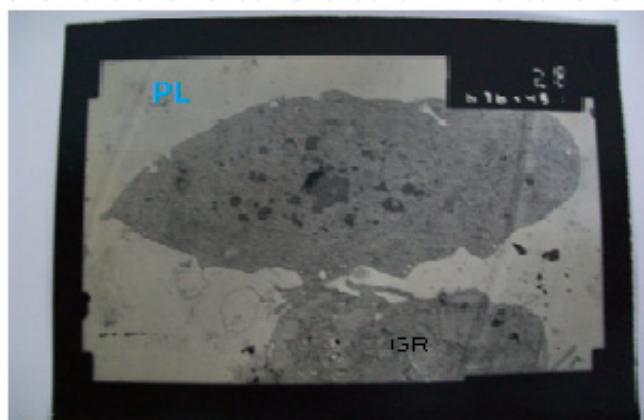**Plate 12.** Pinocytic vesicle formation of PL at 12 h of treatment with *A. conyzoides*

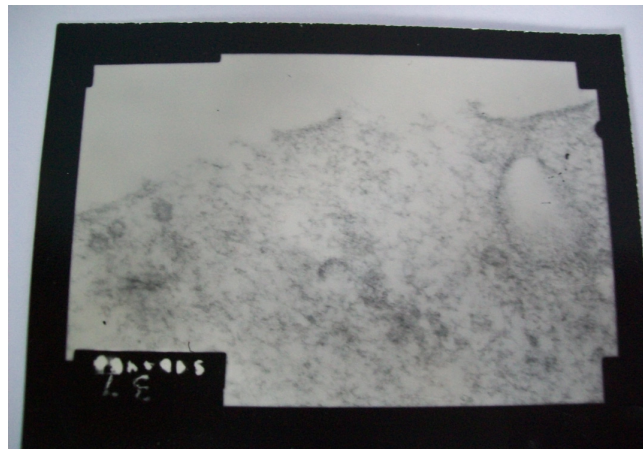**Plate 13.** Cell clumping between PLs and GRs at12 h of treatment with *A. conyzoides*

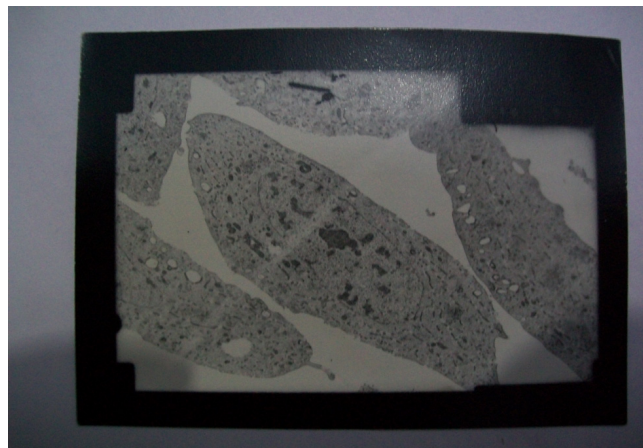**Plate 14.** PM rupture at 12 hr after treatment with *O. sanctum*

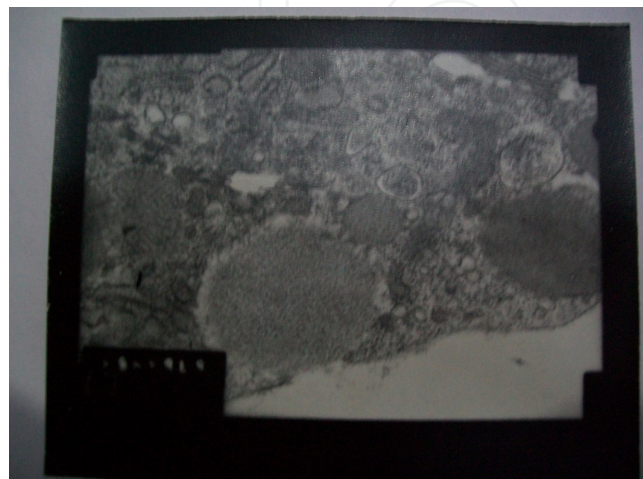**Plate 15.** Normal PLs at 48hr of treatment with *O. sanctum* under TEM

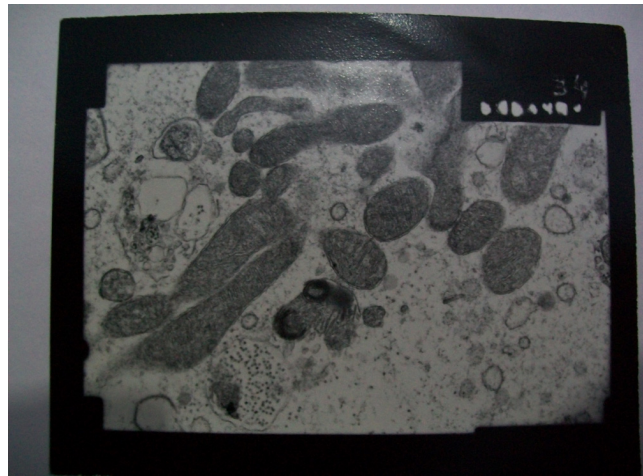**Plate 16.** GR at 1hr of *O. sanctum* treatment under TEM

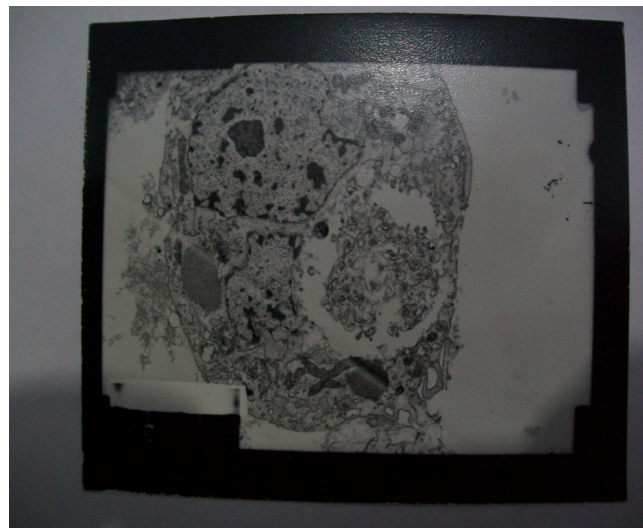**Plate 17.** Mitochondria at 15min of *O. sanctum* treatment

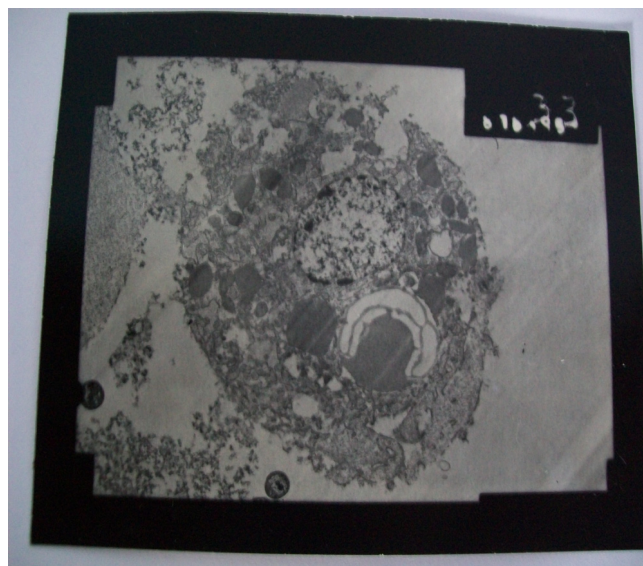**Plate 18.** Granulocyte damage at 15min of *O. sanctum*

Plate 19. Granular material release at 12hr of treatment in *O. sanctum*

Plate 20. GR breakdown at 1hr of treatment in *A. conyzoides A. conyzoides* under TEM
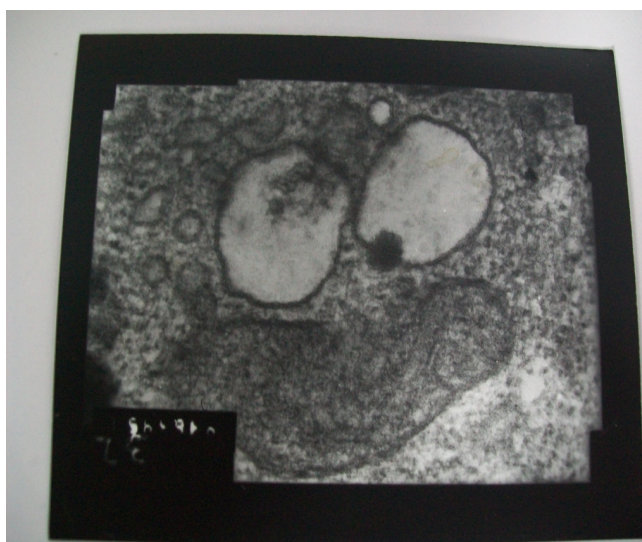

Plate 21. GR mitochondria damage at 12hr of treatment in *A. conyzoides*

5.3. Effect of essential oil on economic characters of silk wrom

Study on impact of essential oils of *O. sanctum* and *A. conyzoides* after topical application at the dosages of 0.2, 0.1, 0.02 and 0.01 μlg^{-1} on 2nd day old *A. assama* fifth instar larvae showed that there was no significant difference on developmental period of fifth instar larvae from that of control in case of treatment of essential oil of *O. sanctum*. A significant decrease in developmental period was caused by essential oil of *A. conyzoides* at the highest dose. Early spinning of larvae after application of essential oil of *A. conyzoides* might be due to its effect on endocrine system. Precocene found in essential oil of *Ageratum* species is known to cause precocious metamorphosis through its action on corpora allata [23,99].

Economic characters of silkworm like cocoon weight and pupal weight of treated larvae did not vary significantly from that of control larvae. But significant decrease of shell weight was recorded after application of essential oil of *A. conyzoides* at all dosages [24]. Reduction in shell

weight might be due to reduction of spinning period causing precocious metamorphosis which might be caused by precocene II found in *A. conyzoides* [99]. Thus in comparison to *A. conyzoides*, *O. sanctum* is a preferable candidates for being used against *E. sorbillan*. However, nutritional indices like efficiency of conversion of ingested food into body matter (ECI), efficiency of conversion of digested food into body matter (ECD), approximate digestibility (AD), consumption index (CI), relative growth rate (RGR) were not found to differ significantly from that of control at lower dosages (0.02, 0.01 µl/g) after treatment of both essential oils of *O. sanctum* and *A.conyzoides* [24]. There are also reports stating beneficial effects of plant products on silk worms which is supportive of use of plant products in sericulture field. Botanicals like *Curcuma longa*, *Phyllanthus ambilica*, *Asparagus racemosus*, *Aegle marmelos*, *Boerhavia diffusa*, *Allium sativum* and *Ocimum basilicum* applied against flacherie infested *Bombyx mori* larvae increased larval weight, cocoon weight, shell weight, silk ratio [100]. Similar studies on beneficial effects of plant products on growth, development and economic characters of silk worms have been carried out by several workers [101-103]. Synergistic actions of plant chemicals might be responsible for growth enhancement of certain insects [104].

Table: Variation of cocoon weight of *A.assama* late instar larvae after treatment with essential oil of plants (P<0.05).

Dose→	0.2µl/g	0.1µl/g	0.02µl/g	0.01µl/g
Plant(oil)↓	Cocoon weight (Mean±SE)	Cocoon weight (Mean±SE)	Cocoon weight (Mean±SE)	Cocoon weight (Mean±SE)
O.sanctum	5.15±.45	4.85±.44	5.13±.44	4.85±.44
A.conyzoides	4.81±.31	5.30±.29	4.81±.31	5.3±.29
Control	5.15±.66	5.15±.66	5.15±.66	5.15±.66

Table: Variation of pupa weight of *A.assama* late instar larvae after treatment with essential oil of plants (P<0.05).

Dose→	0.2µl/g	0.1µl/g	0.02µl/g	0.01µl/g
Plant(oil)↓	Pupal weight (Mean±SE)	Pupal weight (Mean±SE	Pupal weight (Mean±SE	Pupal weight (Mean±SE
O.sanctum	4.69±0.42	4.47±0.46	4.73±0.41	4.47±0.46
A.conyzoides	4.50±0.31	4.96±0.26	4.50±0.31	4.96±0.26
Control	4.75±0.64	4.75±0.64	4.75±0.64	4.75±0.64

Table: Variation of shell weight of *A.assama* after treatment with essential oil of plants (P<0.05).

Dose→	0.2µl/g	0.1µl/g	0.02µl/g	0.01µl/g
Plant(oil)↓	Shell weight (Mean±SE)	Shell weight (Mean±SE)	Shell weight (Mean±SE)	Shell weight (Mean±SE)
O.sanctum	0.34±0.01	0.34±0.04	0.33±0.01	0.34±0.04
A.conyzoides	0.26±0.04	0.29±0.02	0.28±0.01	0.29±0.02
Control	0.32±0.02	0.32±0.02	0.32±0.02	0.32±0.02

Table: Variation of developmental period after treatment with essential oil of plants (P<0.05).

Dose→	0.2µl/g	0.1µl/g	0.02µl/g	0.01µl/g
Plant(oil)↓	Dev period (Mean±SE)	Dev period (Mean±SE)	Dev period (Mean±SE)	Dev period (Mean±SE)
O.sanctum	11.67±.33	11.67±.33	11.33±.33	11.67±.33
A.conyzoides	10±0*	10.67±.33*	11.00±.58*	11.67±.33
Control	11.67±.33	11.67±.33	11.67±.33	11.67±.33

SE→ Standard error; *→ significant at 95% Confidence Interval

Table 4. Variation of cocoon weight of *A. assama* late instar larvae after treatment with essential oil of selected plants (P<0.05)

6. Mechanism of action of botanicals

The efficacy of plant products depend on presence of specific organic compound (s) which may interfere with the body physiology of the target organism. The compounds may belong to different chemical groups of secondary metabolites of plants viz. Alkaloids, phenolics, flavonoids and terpenoids. The ethanolic extract of *O. sanctum* is reported to have greater and broader spectrum of activity against tested organisms [106]. Literature reveals that in comparison to essential oil less works have been carried out to isolate and characterize bioactive compound of *Ocimum sp.* having insecticidal potency through solvent extraction and hence knowledge regarding the use of solvent extracts of *Ocimum* is comparatively less. Petroleum ether and acetone extract of *A. conyzoides* earlier was reported to have juvenile hormone activity against *Culex quinqufasciatus*, *Aedes aegpti* and *Aedes stephensi*. Hexane extract was found effective against *Musca domestica* and methanolic extract caused abnormal development and suppression of population of *Anopheles stephensi* [99,106,107]. Thiophene derivative, a class of compound found in many Asteraceae species has been attributed for the toxic effect of the plant. The hexane fraction of acetone extract of *C. roseus* containing alpha-amyrin is postulated to be active IGR against many pests [63]. Moreira et al. [107] showed the hexane extract of *Ageratum conyzoides* to have insecticidal activity and purified the compounds using IR, 1H NMR, 13 c NMR, HMBC. The compounds were5,6,7,8,3/,4/,5/ heptamethoxy flavone, 5,6,7,8,3/ pentamethoxy-4/,5/-methylene dioxyflavone and coumarin out of which only coumarin showed insecticidal activity against dictyopteran, lepidopteran and dipteran.

The mechanism of toxic effect of essential oil and oil compounds on insect at present is not well known. Insects vary enormously in their response to different essential oils and oil compounds. Essential oils are liquid in room temperature and get easily transformed from a liquid to a gaseous state at room or slightly higher temperature without undergoing decomposition. The quantity of essential oil found in most plants is 1 to 2% but can contain amounts ranging from 0.01 to 10%. Most essential oils comprise of monoterpenes with 10 carbon atoms, sesuiterpenes with 15 carbon atoms and rarely diterpenes or higher terpenes. The most predominant groups are cyclic compounds with saturated and unsaturated hexacyclic or an aromatic system. Bicyclic and acyclic components are also present[2]. Monoterpenes are common essential oil constituents and several hundred naturally occurring monoerpenes are reported. They are biosynthesized from geranyl pyrophosphate of the isoprenoid pathway. These can be classified into two major groups- monoterpene hydrocarbons and oxygenated monoterpenes. Monoterpene hydrocarbons include acyclic aliphatic, monocyclic aliphatic and dicyclic aliphatic while oxygenated monoterpenes include acyclic monoterpenoid, monocyic monoterpenoid and dicyclic monoterpenoids [41]. Some of the major oil constituents of *O. sanctum* are methyl eugenol, caryophyllene, germacrene D, β-elemene, eugenol, caryophyllene epoxide and α-cadinol [24,108,109]. Oil of *A. conyzoides* plant collected from South-East Asian countries mostly contains sesquiterpene Beta-caryophyllene, demethoxyageratochromene and many monoterpenes [24,110]. The monoterpenes in Indian oil of *A. conyzoides* have been reported to be mainly Ocimene (5.3%), alpha-pinene (6.6%), eugenol (4.4%), methyl eugenol (1.8%) and the sesquiterpenes are Beta-caryophyllene(1.9%), Delta- cadinene(4.3%), sesquiphellandrene (1.2%) and caryophyllene

epoxide (0.5%) [62]. Eugenol was earlier reported to be effective against mosquitoes in Michigan [60]. Compounds like coumarin and mainly furanocoumarins can alter the detoxication capability of an organism by reversibly or irreversibly inhibiting cytochrome P450 detoxication enzymes [111-112]. They are also reported to have neurotoxic mode of action probably through binding with different types of octapamine receptors and interference with octapamine activity [113-115] or interference with GABA-gated chloride channels [2,115] or inhibition of achetylcholine esterase [116-118]. Such activity of the oil may be attributed to action of a single major compound or synergistic actions of group of compounds [119].

The root-bark extract of *Zanthoxylum nitidum* having efficacy against rice pest contains several alkaloids [120,121] out of which benzo[c]phenanthridine alkaloids (sanguinarine and chelerythrine) are reported to have the molluscicidal activity [122]. Alkaloid is a large group of plant secondary metabolites and its occurrence has been reported in approximately 20% of all plant species. Most of the alkaloids exert their effect on different region of the nervous system. Different neuroreceptors like alpha, serotonin, muscarinic, nicotinic acetylcholine receptors, adrenergic receptor and enzymes involved in synaptic transmission are some of the target sites of alkaloids. Basic molecular targets are DNA intercalation, protein biosynthesis and membrane stability [123].

The structures of phenolic allelochemicals and their mode of action are diverse [124]. In lepidopteran larvae, phenolic toxicity might occur in the form of oxidative stress [125]. However, *Heliothis virescens* larvae after feeding with high phenolic foliage exhibited improved total Trolox equivalent antioxidant capacity (TEAC) in haemolymph [126]. Therefore it has been proposed that the elevated foliar phenolics in some plants might have beneficial antioxidant properties for herbivorous insects. Flavonoids are known to inhibit cholinesterases [127] and might be responsible for insecticidal action [128]. Various forms of saponins and sterols derived from different plants have been reported to act as insect growth regulator [129].

7. The future of prospects of botanicals in seri-ecosystem

An important essence of integrated pest management is to consider the whole ecosystem as the management unit. A seri-ecosystem should necessarily include not only the sericulture field or the silkworm culture units, but also the agricultural field in the neighbouring areas. A consensus approach is to keep the natural ecosystem largely intact. While attempts are made to control pests and pathogens in the neighbouring agricultural fields, issues regarding their impact on sericulture activities must be taken into consideration or vice-versa. Emphasis is to be given on studies associated with efficacy of plant and plant products against pests and pathogens in the whole management unit, their mode and mechanism of action, identification of target sites and use of resistant strains. Life scientist and chemist need to act coherently to make effective use of botanicals. With the knowledge in hand we hypothesize that holistic approach involving in depth research for using botanical in both sericulture field and the other crop systems in the vicinity might be able to provide a green environment to the silk worms.

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